Possible emissions of N₂O from plants in coniferous-deciduous mixed forests

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Abstract: Aboveground vertical profiles of N₂O concentrations were measured within two natural coniferous-deciduous mixed forests of 1998 and 1999 in Changbai Mountain. Significant high N₂O concentrations were found in six profiles out of twelve profiles. The results showed that high concentrations were 3.03% to 64.9% higher than the "normal concentrations" in these six profiles. Differences between the high concentrations and the "normal concentrations" were statistically significant. The simultaneous occurrence of high concentrations at/nearby the canopy height and normal concentrations at the trunk space height indicated an efflux of N₂O from foliage to atmosphere. This study afforded evidence supporting that plant *per se*, besides forest soil, was an important source of atmospheric N₂O in a forest ecosystem.

Key words: Nitrous oxide; Concentration profile; Plant; Forest ecosystem

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Introduction

Nitrous oxide (N2O) is a greenhouse gas with a global warming potential per unit mass that is 120-330 times greater than CO₂ over next 100 years. The increased N₂O in the atmosphere will also lead to increased ultraviolet radiation through the catalytic destruction of stratospheric ozone. The current growth rate of its atmospheric concentration is 0.8 μL L¹·a⁻¹. Provided that N₂O emissions are held constant at current levels, its concentration will rise to about 400 µL L⁻¹ over next several hundred years. This would increase its incremental radiative forcing by a factor of four over its current level (IPCC 1995). There is an increased need to study the natural sources of N₂O because current estimates of N₂O inputs to the atmosphere is only about half of the flux required to balance the calculated global destruction rate (Kim and Craig 1993). One of reasons for this imbalance should be some significant sources are undiscovered.

According to the current knowledge, biogenic nitrous oxide is produced only by soil microbial processes. Denitrification and nitrification are considered to be the most important ones. Up to 90% of the

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Received date: 2000-10-03 Responsible editor: Song Funan global N2O production was believed to arise from soil biological processes (Bouwman 1990). However, in recent years, some laboratory studies have led to a clue that plant may play an important role in N2O emission and uptake. Lensi & Chalamet (1981) reported that plant could take up and remove N2O from air. This group also reported that ¹⁵N₂O was taken up by maize leaves and metabolized as a source of N (Grundmann et al. 1993). Plants can emit N2O when stressed was reported by Chen et al. (1990). Studies with germ-free soybean plants in closed chamber were conducted in the condition of C₂H₂ addition, and N₂O emissions were observed (Huang et al. 1992: Chen et al. 1995). However, no evidence has been reported till now that plant can emit N2O under natural conditions.

Vegetation is in the largest possession of biomass in biosphere and forest ecosystems play an important role in the biogeochemistry cycling of nitrogen and carbon. Therefore, discernment of plant's role in N₂O emission/uptake under natural conditions would provide another component to our understanding of atmospheric N₂O budget imbalance.

We postulated that significant amounts of N_2O might be released from foliage of plants in a forest ecosystem. Therefore, the high N_2O concentration would appear near the canopy height in a stable meteorologic condition. In this study, our objective was to verify the existence of significant high/low concentration near the canopy height.

Methods

Experiment Sites

The study was conducted at two sites located respectively in two natural coniferous-deciduous mixed

forests in Changbai Mountain Biosphere Reserve, Jilin Province, China (42°24'N, 128°28'E). The site 1 is a 290-year-old primary broad-leaved Korean pine mixed forest with an average canopy height of 28 m and its crowns reach down to 7 m. The site 2 is an 11-year-old secondary broad-leaved Korean pine mixed forest with an average canopy height of 3.2 m. The canopy densities at site 1 and site 2 are 0.8 and 0.7 respectively. The main standing trees in these forests are *Pinus koraiensis*, *Tilia amurrensis*, *Fraxinus mandshurica*. Soils of two sites are mountain dark brown forest soil. Mean annual precipitation is 800 mm. Mean annual temperature is 3.3 °C. Soil pH is 5.67/4.75 (H₂O/KCI).

Instrumentation

A 61.5-m and a 5-m-height towers were set up in site 1 and site 2, respectively. The inlet ends of air sampling tubes (stainless steel, with the internal diameter of 5.0 mm) were fixed onto towers at different heights (shown in Fig. 2 & 3). The outlet ends were led to air samplers 10 m away from towers on the ground.

Two steps were operated in a sampling procedure (Fig.1). The first step was shown as state A in Fig.1. In this state, Outlet ends were connected to a vacuum pump. The vacuum pump and the multi-port valve were turned on. Flow rate of air within each sampling tube was adjusted to 200 mL/min by individual flow-meter. Therefore, the sampling tubes were flushed by ambient air coming from different heights of tower. The state A was lasted for 25 min for the purpose of replacing completely the inside air

of sampling tubes by ambient air from different heights. Thereafter, the second step (shown as state B in Fig.1) was operated immediately. The multi-port valve was turned off. The outlet ends were disconnected from the vacuum pump and connected to empty samplers. The sampler is a kind of 500-mL plastic bag with inside subatmospheric pressure when it is empty. The multi-port valve was turned on, and the air within sampling tubes flowed into the empty samplers. The air samples in each sampler were transferred to 500-mL air storage bags (Guangming Chemical Engineering Research Institute, Dalian, CHINA) by connecting samplers to storage bags, and pressing samplers.

Analysis of N2O Concentrations

Replicated subsamples (1 mL) were drawn from storage bags and analyzed for N_2O concentration by GC. A Shimazu GC-14A was operated with an electron capture detector (ECD) at 300 °C, a Porapak-Q precolumn (1 m \times 0.3 cm) and an analytical column (2 m \times 0.3 cm) at 60 °C, and a carrier gas (N_2) at the flow rate of 60 mL·min⁻¹. A 10-port valve permitted the heavier water vapor and other heavier components to be back-flushed from the precolumn at 0.98 min after injection of samples, whereas N_2O were separated on the analytical column.

The quantification of the N₂O concentrations of air samples was based on comparing their integrated peak areas of GC chromatograms with those of two standard samples (0.303 and 0.959 µL·L⁻¹, provided by Chinese Research Center of Standard Substances).

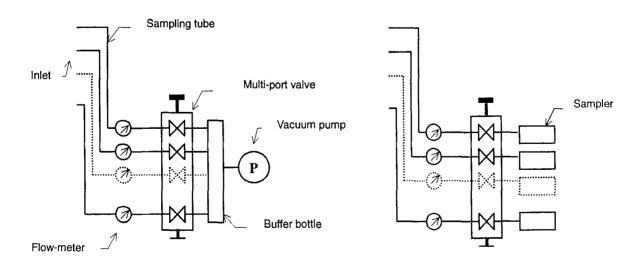


Fig.1 Schematic representation of air synchronizing sampling instrumentation.

Left: State A; Right: State B.

Statistical analysis

N₂O concentrations of air samples at various

heights were subjected to a two-way analysis of variance to test for significant difference by SPSS 6.0

(SPSS Inc. 1993).

Results

Aboveground vertical profiles of N_2O concentrations were measured four times in site 1 and twice in site 2 in summer of 1998, six times in site 1 in summer of 1999. Out of these 12 measurements, there were six measurements in which the statistical significant differences among N_2O concentrations at different heights were found. These six cases of measurements were described in this paper as Profile A, B, C, D, E and F.

N₂O concentration profiles from Site 1

Profile A (9:45 on September 20, 1998)

The vertical concentration profile of N_2O in Site 1 at 9:45 on September 20, 1998 is shown in Fig.2 (profile A). Profile A was characterized by a significant high concentration at the height of 15.75 m. The differences between this high concentration and any concentrations at other heights were statistically significant (P<0.01). The high concentration, 0.382 μ L·L⁻¹, was 24.8% higher than the minimum concentration (0.306 μ L·L⁻¹, at 61.75 m) in this profile.

Profile B (10:06 on Sept. 17, 1999), profile C (10:36 on Sept. 17, 1999) and profile D (14:20 on Sept. 17, 1999)

Other three profiles of N_2O concentration within stand of site 1 were measured on Sept. 17, 1999 (see profile B, C and D in Fig. 2). One significant high concentration was observed at heights of 7.50, 32.00 and 15.75 m respectively in each profile. The differences between each high concentration in a profile and the other concentrations were statistically significant.

N₂O concentration profiles from Site 2

Profile E (10:00 on July 2, 1998)

Fig. 3 shows the synchronous N₂O concentrations at different heights at 10:00 on July 2, 1998. Two high N_2O concentrations, 0.322 and 0.323 $\mu L \cdot L^{-1}$, were found at heights of 3.20 and 3.30 m. Three "normal concentrations" 0.309, 0.308 and 0.309 µl·[' were found at heights of 0.40, 2.00 and 3.50 m. The "normal concentrations" called here was similar to the atmospheric background concentration. The high concentrations were about 4.5% higher than the normal concentrations and the difference between any one of a high concentration and anyone of a normal concentration was statistically significant (P<0.05). In profile E, the high N₂O concentrations were found to appear at average canopy height, but the "normal concentrations" at two ranges of heights: the trunk space height and the height above average canopy height.

Profile F (15:20 on August 16, 1998)

In profile F (Fig. 3), two significant high N_2O concentrations (0.306 and 0.320 $\mu\text{L}\cdot\text{L}^{-1}$) at 3.40 m height and on the soil surface were found, respectively. The 3.40 m height was just 10 cm higher over the upper-boundary of canopy. However, two "normal concentrations" (both 0.297 $\mu\text{L}\cdot\text{L}^{-1}$) were found at heights of 2.80 m and 4.30 m, which were located at the trunk space height and at the height above upper-boundary of canopy. The two high concentrations were 3.03% and 7.74% higher than the "normal concentrations". The difference between a high concentration and a "normal concentration" was statistically significant (P<0.05).

N₂O concentration profiles on open ground

Aboveground N_2O concentrations at six different heights up to 4 m were also measured on an open ground at 15:20 on September 20, 1998. The open ground was adjacent to the stand of site 2. Two years ago, it once was the same secondary forest as site 2. But the trees were felled since then each year. The results showed there was no significant difference between N_2O concentrations at different heights in the profile.

Discussion and conclusions

In this study, the experiments were conducted with a synchronizing sampling method, which allowed the synchronous sampling of air at various heights from ground to heights above canopy in a forest ecosystem. When the N₂O concentrations of air samples had been quantified with GC, vertical N₂O concentration profiles in a forest stand could be acquired.

Twelve profiles of N₂O concentration were measured in two mixed Korean pine forests in 1998 and 1999 at site 1 and site 2. Among these profiles, there were six cases of measurements (see profiles A, B, C, D, E and F) in which significant high N₂O concentrations were found. These high N₂O concentrations were 3.03% to 64.9% higher than the "normal concentrations" in each profile. Differences between the high concentrations and the "normal concentrations" were statistically significant. Firstly, this result suggested that it was a feasible approach to describe the vertical N₂O concentration profile in a forest with the combination of synchronizing sampling method and concentration analysis with GC. Secondly, the repetitive observation of significant high N2O concentration in these six profiles indicated the occurrence of high N₂O concentration was a repeatable phenomenon, rather than an uncommon case.

From results at site 1, the significant high N₂O concentrations in profile A, B, C and D appeared at heights of 15.75, 7.50, 15.75 and 32.00 m, respec-

tively. All of these high concentrations were located within or near the range between canopy's upper boundary (Ca. 31 m) and lower boundary (Ca. 7 m). In profiles from site 2, the high N_2O concentrations

appeared at only about 10-20 cm above the upper boundary of the canopy. The phenomenon that significant high N_2O concentration appeared within or near the canopy height was hitherto not reported.

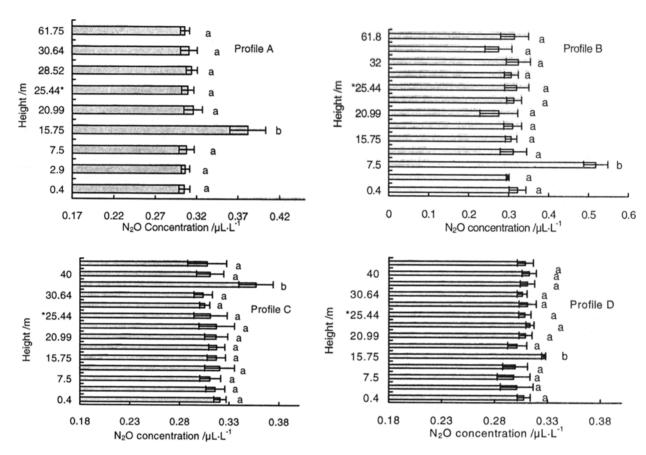


Fig.2 Above ground N₂O concentration profile in the primary broadleaved-Korean pine mixed forest (site 1).

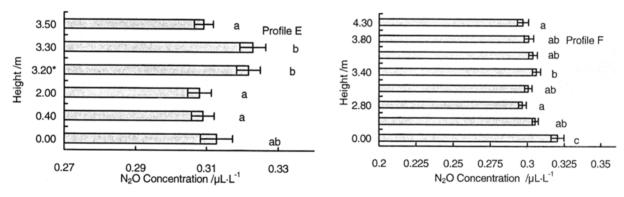


Fig.3 Above ground N₂O concentration profile in the secondary broadleaved-Korean pine mixed forest (site 2).

 N_2O was emitted from canopy foliage can be deduced from this phenomenon. The observed high concentrations at/near the canopy height indicated there was an outward flux of N_2O from canopy to atmosphere. This efflux was not driven directly by N_2O production in forest soil, because no high N_2O concentration was found at the trunk space heights. Provided that N_2O was solely emitted from soil instead of from plant, according to the law of gas diffu-

sion, the aboveground N_2O concentrations should be decreased with the increase of vertical height in a stable meteorological condition. Therefore, the joint occurrence of the high concentrations at the canopy height and the normal concentrations at the trunk space height can be only explained by that the foliage of plants emit N_2O . That is to say, the high concentration at canopy height did not ascribe to N_2O emission from soil surface, but to N_2O emission from

plant.

The N₂O efflux from plant can be explained with two possible mechanisms. The first one is that the high N₂O concentration at or near the canopy height is derived only from microbial processes in forest soil. The N₂O is produced within soil and dissolved in soil solution. The dissolved N2O is absorbed by plant roots along with water, and then transported to leaves by transpiration. The emitted N₂O is derived from soil. Observations of Chang et al. (1998) provided evidence that the plants did not generate the N₂O directly, but merely conveyed N₂O produced in the soil. Yang et al. (1995) reported that N₂O emissions from several coniferous and broad-leaved tree species were increased with increasing temperature and respiration rate. Stomata were probably the main pathway for N₂O emission. The second mechanism is that N₂O is produced from physiological metabolism of plant itself. Huang Guohong et al. (1992) observed the N₂O emission from germ-free sovbean plant when C₂H₂ was added. This result indicated the possibility of N₂O producing mechanism by plant itself because no microorganisms existed in the system. However, which one is the definite mechanism or whether these two mechanisms are in operation at the same moment? Further researches, such as method with N isotope technology, are needed to distinguish the two mechanisms.

A closed chamber technique is widely applied to the measurement of N₂O exchange between soil and atmosphere. Based on the results from the measurements with closed chamber method, Kerileman and Bouwman (1994) estimated that the N2O emission from closed tropical forests, open tropical forests and temperate forests were 2.3, 1.0 and 0.5 Tg·a⁻¹ respectively. But the total amount of N₂O emission from terrestrial ecosystems was estimated only as 6.6 Tg a 1. Castro et al. (1993) estimated that soils in spruce-fir forests in the northeastern United States released about 0.02 to 0.08 kg N₂O-N hm⁻²·a⁻¹. However, the results indicated the N₂O emissions solely from soil surface. If the plant is proved to be able to emit N₂O, the closed chamber method may result in underestimation of actual fluxes from a forest ecosystem. This may be one of possible reasons for the unbalanced global N₂O budget. More attentions, therefore, should be paid to the plants in the researching of N₂O emission from an ecosystem. However, compared to the chamber technique, the synchronizing sampling method led to much less disturbance to the gas fluxes. Thereafter, the profiles will provide the opportunity to investigate gas exchanges between plant/soil and atmosphere in natural condition.

The occurrence of high concentrations at or near

the canopy heights in N₂O concentration profiles within the forest ecosystem was reported for the first time. This study provided the direct evidence that plant in natural condition is able to emit N₂O. However, the interpretation of gas concentration profiles within canopies can only be used to reveal qualitatively the role of plant as source or sink of N₂O. Although our results showed that the vertical N2O concentration fluctuation in the forest ecosystem was detectable by GC analysis, eddy correlation methods when further developed with cheap, robust sensors for N₂O will be an important tool for quantitative measuring N₂O exchange. It will offer exciting opportunities to further our understanding of biological mechanisms controlling gas exchange when experiments are amalgamated with parallel physiological measurements.

References

Bouwman, A. F. 1990. Exchange of greenhouse gases between terrestrial ecosystems and the atmosphere. In: Bouwman A. F. (Ed). Soils and the Greenhouse Effect. Wiley, Chichester, 61-127.

Castro, M.S. *et al.* 1993. Exchange of nitrous oxide and methane between the atmosphere and soils in spruce-fir forests in the northeastern United States [J]. Biogeochemistry, **18**(3): 119-135.

Chang, C. et al. 1998. Nitrous oxide emission through plants [J]. Soil SCI. SOC. AM. J., 62: 35-38.

Chen Guanxiong *et al.*, 1990. Investigation on emission of N₂O by plant [J]. Chin. J. Appl. Ecol. 1 (1): 94-96.

Chen Xin *et al.* 1995. A preliminary research on the effect of nitrogen and phosphorus supply on N₂O emission by crops [J]. Chin. J. Appl. Ecol., 6 (1): 104-105.

Grundmann, G.L.,R. Lensi & A. Chalamet, 1993. Delayed NH₃ and N₂O uptake by maize leaves [J]. New Phytol. **124**: 259-263.

Huang Guohong *et al.* 1992. Investigation on emission of N_2O by aseptic soybean plant [J]. Acta Botanica Sinica (in Chinese), **34** (1): 835-839.

IPCC. 1995. Summary for Policymakers: The Science of Climate Change - IPCC Working Group I.

Kim K. -R. and Craig H., 1993. Nitrogen-15 and oxygen-18 characteristics of nitrous oxide: a global perspectives [J]. Sciences **262**, 1855-1857.

Kreileman G.J.J. and A.F. Bouwman (1994) Computing land use emissions of greenhouse gases [J]. Water, Air and Soil Pollution 76, 231-258.

Lensi, R. &A. Chalamet, 1981. (Absorption of nitrous oxide by shoots of maize). In French [J]. Plant Soil., 59: 91-98.

Yang Sihe *et al.*, 1995. N₂O emission from woody plants and its relation to their physiological activities [J]. Chin. J. Appl. Ecol., **6**(4): 337-340.